Asian Journal of Pharmaceutical Education and Research
Vol -12, Issue-2, April- June 2023
ISSN:2278 7496

RESEARCH ARTICLE Impact Factor: 7.014

FORMULATION & EVALUATION OF PHYTOSOME OF *PRUNELLA VULGARIS* FOR ANTI DIABETIC ACTIVITY

Doli Kumari Singh*, Sachin K. Jain
Oriental College of Pharmacy and Research, Oriental University, Indore (M.P.)

*Corresponding Author's E mail: tomardolly1@gmail.com
Received 16 Feb. 2023; Revised 26 Feb. 2023; Accepted 11 Mar. 2023, Available online 15 Apr. 2023.

Cite this article as: Singh DK, Jain SK. Formulation and Evaluation of Phytosome of *Prunella Vulgaris* for Anti Diabetic Activity. Asian Journal of Pharmaceutical Education and Research. 2023; 12(2): 46-56.
https://dx.doi.org/10.38164/AJPER/12.2.2023.46-56

ABSTRACT

Diabetes mellitus is a set of disorders that cause hyperglycemia and glucose intolerance as a result of a lack of insulin, defective insulin function, or both. Diabetes can further give rise to other diseases. So, the aim of this study is the formulation of phytosome by use of bioactive constituent of prunella vulgaris and its evaluation for anti diabetic effect. The plant material was obtained and subjected to extraction. Further the qualitative & quantitative estimation of phytochemicals was performed. The phytosomes were formulated & evaluated for every parameter along with anti diabetic activity. The results showed that the percentage yield of hydroalcoholic extract of *Prunella vulgaris* exhibited higher yield 7.56% followed by pet. ether extract of 2.78%. The total phenolic and flavonoid contents of *Prunella vulgaris* showed the content value 0.475mg GAE/100mg extract and 0.846mg QAE/100mg extract respectively. The entrapment efficiency of the phytosomes was found in the range of 65.52 to 75.65%. Particle size of all formulations found within range 236.65-365.45nm. Formulation F10 was found best. The IC 50 value for phytosome by alpha amylase assay was found to be 231.22 (µg/ml). The first order was found to be maximum that is 0.988 hence indicating drug release from formulations was found to follow First Order release kinetics. Results of stability studies clearly indicates that optimized batches of phytosomes were stable over the chosen temperature and humidity conditions up to 3 months as were found no significant variation in physical appearance and % drug content. Thus, it can be concluded that formulated F10 phytosome exhibits all ideal characteristics & can be used to treat diabetes.

Keywords: Anti diabetic, Herbal Medicine, Phytosome, *Prunella vulgaris.*

INTRODUCTION

Diabetes mellitus is a set of disorders that cause hyperglycemia and glucose intolerance as a result of a lack of insulin, defective insulin function, or both. Failures in the regulatory systems for the storage and mobilisation of metabolic fuels, such as carbohydrate, lipid, and protein catabolism and anabolism, are caused by incorrect insulin synthesis, insulin action, or both, resulting in such challenges. There are two types of diabetes mellites [1-3].

In type I diabetes melites the CD4+ and CD8+ T lymphocytes, as well as macrophages invading the islets, destroy insulin-producing cells in the pancreas, resulting in Type 1 Diabetes. The autoimmune death of pancreatic -cells causes a lack of insulin secretion, leading to the metabolic abnormalities associated with T1DM. In T1DM patients, the function of pancreatic -cells are aberrant, and there is excessive production of glucagons in addition to the loss of insulin secretion. Insulin insufficiency results in uncontrolled lipolysis and elevated plasma levels of free fatty acids, which prevents glucose from being used in peripheral tissues such as skeletal muscle [4,5].

In type 2 diabetes there is impaired insulin secretion through a dysfunction of the pancreatic β-cell, and impaired insulin action through insulin resistance. Hepatic insulin resistance is the driving force of hyperglycemia in type 2 diabetes because it occurs in the context of hyper insulinemia, at least in the early and intermediate phases of the illness [6].

Phytochemicals are bioactive polyphenolic compounds naturally found in plants that have been studied extensively due to their potential medicinal and nutritional benefits to humans Phytochemicals are categorized into three major categories based on their structural elements: terpenoids, alkaloids and polyphenolic substances. Numerous flavor and aromatic molecules are trepeniods, including menthol, linalool, geraniol, and caryophyllene, while catechols, lignins, tannins, stilbenes, and flavonoids are phenolic compounds [7-9].

A number of chief constituents of herbal medicine are easily soluble in water (glycoside, flavonoid); however, these constituents are bounded in their potency because they may be partially soluble or hydrophobic in nature, so when applied topically shows less therapeutic efficacy [10,11].

Numerous efforts have been put forward to enhance the bioavailability of such drug by formulating them to target drug delivery system such as phytosomes and liposomes are good options Phytosomes means herbal drug loaded in vesicles, which is available in the Nano form. The phytosome provide an envelope, like coating around the active constituent of drug and due to this the chief constituent of herbal extract remains safe from degradation by digestive secretion and bacteria. Phytosome is effectively able to absorb from a water loving environment into lipid loving environment of the cell membrane and finally reaching to blood circulation. This study deals with the evaluation of *Prunella vulgaris* plant for its antidiabetic activity through a phytosomal approach [12-14].

MATERIALS AND METHODS

Material

Hydrochloride, Disodium Hydrogen Phosphate, Di potassium Hydrogen Orthophosphate, Sodium Chloride, Dichloromethane were obtained from S.D fine chemicals Mumbai. Methanol, Ethanol and

Chloroform were obtained from Qualigen chemicals Mumbai. Cholesterol and lecithins obtained from RFCL Ltd. Delhi.

Collection of Plant material

The plants have been selected on the basis of its availability and Folk use of the plant. Aerial part of Prunella vulgaris were collected from local area of Bhopal in the month of April, 2021.Drying of fresh plant parts were carried out in sun but under the shade. Dried aerial part of Prunella vulgaris were preserved in plastic bags and closed tightly and powdered as per the requirements.

Methods

Extraction of plant

56.84 gm of dried powdered aerial part of *Prunella vulgaris* were coarsely powdered and subjected to extraction with petroleum ether by maceration. The extraction was continued till the defatting of the material had taken place. Defatted dried powdered aerial part of *Prunella vulgaris* has been extracted with hydroalcoholic solvent (ethanol: water: 75:25) using maceration method for 48 hrs, filtered and dried using vacuum evaporator at 400C.

Phytochemical screening

Phytochemical examinations were carried out for the extract as per the standard methods [15].

Quantitative estimation of bioactive compounds

Total Phenolic content estimation

The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method. 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 5- 25µg/ml was prepared in methanol. 10mg of dried extracts of were dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this solution was used for the estimation of phenol. 2 ml of each extract or standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 15 min for colour development. The absorbance was measured at 765 nm using a spectrophotometer [16].

Total flavonoids content estimation

Determination of total flavonoids content was based on aluminium chloride method. 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol. 10mg of dried extracts of were dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this solution was used for the estimation of flavonoid. 1 ml of 2% $AlCl_3$ methanolic solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; absorbance was measured at 420 nm[17].

Formulation development of Phytosomes

The complex was prepared with phospholipids: Cholesterol and *Prunella vulgaris* in the ratio of 1:.5:1, 1:1:1, 2:1.5:1, 2:2:1 respectively. Weight amount of extract and phospholipids and cholesterol were placed in a 100ml round-bottom flask and 25ml of dichloromethane was added as reaction medium. The mixture was refluxed and the reaction temperature of the complex was controlled to 50°C for 3 h. The resultant clear mixture was evaporated and 20 ml of n-hexane was added to it with stirring. The precipitated was filtered and dried under vacuum to remove the traces amount of solvents. The dried residues were gathered and placed in desiccators overnight and stored at room temperature in an amber-colored glass bottle [18].

Table 1: Different formulations of Phytosomes

Formulation	Ratio of Phospholipids and Cholesterol	Extract Concentration (%)	Dichloromethane Concentration
Optimization of Phospholipids and Cholesterol			
F1	1:05	1	25
F2	**1:1**	**1**	**25**
F3	1:1.5	1	25
F4	1:2	1	25
Optimization of Drug Concentration			
F5	1:1	0.5	25
F6	**1:1**	**1.0**	**25**
F7	1:1	1.5	25
F8	1:1	2.0	25
Optimization of solvent concentration			
F9	1:1	1.0	10
F10	**1:1**	**1.0**	**25**
F11	1:1	1.0	50
F12	1:1	1.0	75

Characterization of Phytosomes

Entrapment efficiency

Phytosome preparation was taken and subjected to centrifugation using cooling centrifuge (Remi) at 12000 rpm for an hour at 4 [19].

The clear supernatant was siphoned off carefully to separate the non-entrapped flavonoids and the absorbance of supernatant for nonentrapped Prunella vulgaris extract was recorded at λmax 420.0 nm using UV/visible spectrophotometer (Labindia 3000+). Sediment was treated with 1ml of 0.1 % Triton x 100 to lyse the vesicles and diluted to 100 ml with 0.1 N HCl and absorbance taken at 420.0 nm. Amount of quercetin in supernatant and sediment gave a total amount of Prunella vulgaris extract in 1 ml dispersion.

Particle size and size distribution

The particle size, size distribution and zeta potential of optimized phytosomes formulation were determined by dynamic light scattering (DLS) using a computerized inspection system (Malvern Zetamaster ZEM 5002, Malvern, UK). The electric potential of the phytosomes, including its Stern layer (zeta potential) was determined by injecting the diluted system into a zeta potential measurement cell [20].

Transmission electron microscopy

Surface morphology was determined by TEM, for TEM a drop of the sample was placed on a carbon-coated copper grid and after 15 min it was negatively stained with 1% aqueous solution of phosphotungustic acid. The grid was allowed to air dry thoroughly and samples were viewed on a transmission electron microscopy (TEM Hitachi, H-7500 Tokyo, Japan).

In vitro anti-diabetic activity of phytosome using inhibition of alpha amylase enzyme

Preparation of standard: 10 mg acarbose was dissolved in 10 ml methanol, and various aliquots of 100- 500µg/ml were prepared in methanol.

Preparation of sample: 10 mg of phytosome was extracted with 10 ml methanol. 500 µl of this solution was used for the estimation of enzyme inhibition.

Method: A total of 500 µl of test samples and standard drug (100-500µg/ml) were added to 500 µl of 0.20 mM phosphate buffer (pH 6.9) containing α-amylase (0.5mg/ml) solution and were incubated at 25°C for 10 min. After these, 500 µl of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube. The reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped with 1.0 ml of 3, 5 dinitrosalicylic acid colour reagent. The test tubes were then incubated in a boiling water bath for 5 min, cooled to room temperature. The reaction mixture was then diluted after adding 10 ml distilled water and absorbance was measured at 540 nm. Control represents 100% enzyme activity and were conducted in similar way by replacing extract with vehicle.

In vitro dissolution rate studies

In vitro drug release of the sample was carried out using USP- type I dissolution apparatus (Basket type). The dissolution medium, 900 ml 0.1N HCl was placed into the dissolution flask maintaining the temperature of 37±0.5°C and 75 rpm. 10 mg of prepared phytosomes was placed in each basket of dissolution apparatus. The apparatus was allowed to run for 8 hours. Sample measuring 3 ml were withdrawn after every interval (30 min, 1 hrs, 2 hrs, 4 hrs, 6 hrs, 8 hrs, and 12 hrs.) up to 12 hours using 10 ml pipette. The fresh dissolution medium (37^0C) was replaced every time with the same quantity of the sample and takes the absorbance at 256.0 nm using spectroscopy.

Mathematical treatment of *in-vitro* release data: The quantitative analysis of the values obtained in dissolution/release tests is easier when mathematical formulas that express the dissolution results as a function of some of the dosage forms characteristics are used. Zero order, first order, Higuchi & Korsmeyer peppas model was evaluated [21].

Stability studies of optimize Phytosomes Formulation

The prepared phytosomes subjected to stability studies at 40±2°C/75±5% RH and 30±2°C/60±5% RH as per ICH guidelines for a period of 3 months. Samples were withdrawn at 1 month time intervals and evaluated for physical appearance and drug content [22].

RESULTS & DISCUSSION

The percentage yield of hydroalcoholic extract of *Prunella vulgaris* exhibited higher yield 7.56% followed by pet. ether extract of 2.78%. Due to these results, can be concluded as *Prunella vulgaris* hydroalcoholic extract possess more phytoconstituents as compare to pet. ether extract. The hydroalcoholic extract of *Prunella vulgaris* had revealed the presence of diterpenes, tannins, protein, flavonoids, phenols, carbohydrates and saponins. Glycosides and alkaloids were found to be absent in *Prunella vulgaris*. The total phenolic and flavonoid contents of *Prunella vulgaris* showed the content value 0.475mg GAE/100mg extract and 0.846mg QAE/100mg extract respectively

The entrapment efficiency of the phytosomes was found in the range of 65.52 to 75.65%. Particle size of all formulations found within range 236.65-365.45nm. Concentration of lipid has shows significant impact on size of phytosomes. Formulation F10 was found best one which is further evaluated for drug release study, transmission electron microscopy (TEM), and stability studies. When the regression coefficient values of were compared, it was observed that 'r^2' values of First Order was maximum i.e. 0.988 hence indicating drug release from formulations was found to follow First Order release kinetics.

Results of stability studies clearly indicates that optimized batches of phytosomes were stable over the chosen temperature and humidity conditions up to 3 months as were found no significant variation in physical appearance and % drug content.

Table 2: % Yield of *Prunella vulgaris*

S. No.	Solvents	% Yield (w/w)
1	Pet ether	2.78%
2.	Hydroalcoholic	7.56%

Table 3: Phytochemical screening of extract of *Prunella vulgaris*

S. No.	Constituents	Hydroalcoholic extract
1.	**Alkaloids**	
	Wagner's Test	-ve
	Hager's test	-ve
2.	**Glycosides**	
	Legal's test	-ve
3.	**Flavonoids**	
	Lead acetate	+ve
	Alkaline test	+ve
4.	**Phenolics**	
	Ferric Chloride Test	+ve
5.	**Proteins**	
	Xanthoproteic test	+ve
6.	**Carbohydrates**	
	Fehling's test	+ve
7.	**Saponins**	
	Froth Test	+ve
	Foam test	+ve
8.	**Diterpenes**	
	Copper acetate test	-ve
9.	**Tannins**	
	Gelatin Test	+ve

Table 4: Total phenolic and total flavonoid content of *Prunella vulgaris*

S. No.	Extract	Total Phenol (mg/100mg)	Total Flavonoids (mg/100mg)
1.	Hydroalcoholic extract	0.475	0.846

Table 5: Particle size and entrapment efficiency of drug loaded Phytosomes

Formulation Code	Particle size (nm)	Entrapment Efficiency (%)
F1	365.45	65.58
F2	285.45	72.23
F3	312.25	69.98
F4	342.12	67.74
F5	298.45	68.85
F6	245.65	73.12
F7	285.65	69.12
F8	296.65	65.85
F9	265.74	65.52
F10	236.65	75.65
F11	245.65	67.74
F12	262.32	68.85

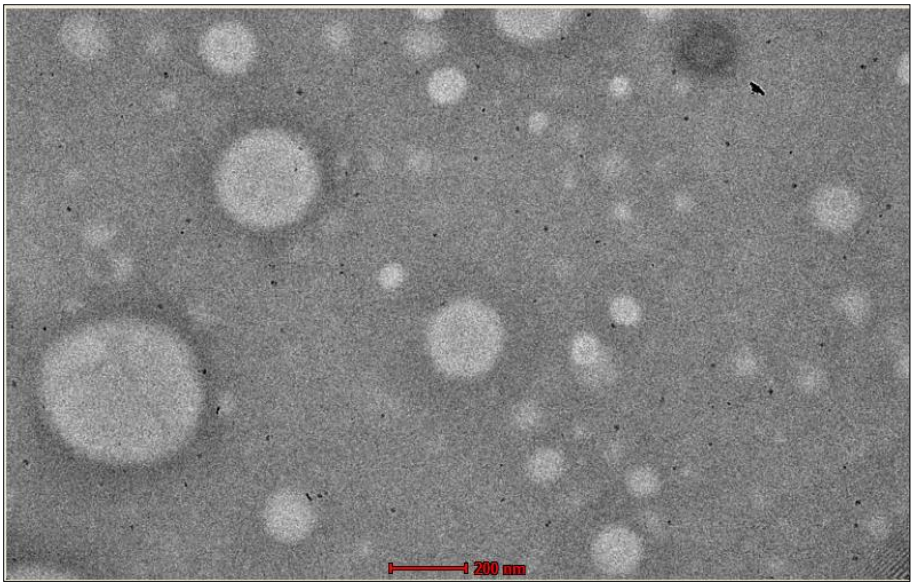

Figure 1: TEM image of phytosomes

Table 6: % Inhibition of acarbose and prepared phytosomes of *Prunella vulgaris*

S. No.	Concentration (µg/ml)	% Inhibition	
		Acarbose	Phytosomes
1	100	52.32	34.51
2	200	69.98	48.02
3	300	73.32	54.65
4	400	79.95	62.75
5	500	89.85	70.87
IC_{50} (µg/ml)		**28.58**	**231.22**

Table 7: *In-vitro* drug release data for optimized formulation F10

Time (h)	Square Root of Time(h)$^{1/2}$	Log Time	Cumulative*% Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
0.5	0.707	-0.301	25.65	1.409	74.35	1.871
1	1	0	36.65	1.564	63.35	1.802
2	1.414	0.301	48.85	1.689	51.15	1.709
4	2	0.602	72.23	1.859	27.77	1.444
6	2.449	0.778	89.98	1.954	10.02	1.001
8	2.828	0.903	96.65	1.985	3.35	0.525
12	3.464	1.079	99.45	1.998	0.55	-0.260

Table 8: Regression analysis data of optimized formulation F10

Batch	Zero Order	First Order	Higuchi	Korsmeyer Peppas
	R^2	R^2	R^2	R^2
F10	0.949	0.988	0.949	0.982

CONCLUSION

Phytosomes are a novel type of delivery system for herbal medicines and have been shown to be effective in delivering active compounds to target cells for various therapeutic effects. *Prunella vulgaris* is a medicinal herb that has been used for centuries to treat a variety of ailments, including diabetes. It is rich in a variety of bioactive compounds, such as polyphenols, flavonoids, and other antioxidants, which may be responsible for its anti-diabetic activity. In conclusion, in this study, the combined hydroalcoholic extract of *Prunella vulgaris* in ratio of 1:1:1 found to exhibit significant results. The entrapment efficiency of the phytosomes was found in the range of 65.52 to 75.65%. Particle size of all formulations found within range 236.65-365.45nm. Concentration of lipid has shows significant impact on size of phytosomes. The maximum percentage drug release and minimum Particle size was found in formulation F10 was select as optimized formulation. *In-vitro* studies revealed that phytosomes showed control release of phytoconstituents. Hence, phytosomal formulation of this herbal drug combination can be used for clinical application to enhance the therapeutic effect.

REFERENCES

1. Sicree R, Shaw J, Zimmet P. The global burden. Diabetes and impaired glucose tolerance. Prevalence and projections. In: Gan D, editor Diabetes atlas. 3rd ed. Brussels: International Diabetes Federation; 2006. p. 16-103.
2. Shillitoe RW. Psychology and diabetes: psychosocial factors in management and control; 1988.
3. Votey SR, Peters AL. Diabetes mellitus type 2. A review; 2004 [cited July 2006]. Available from: http://www.emedicine.com/emerg/topic133.htm.
4. Al Homsi MF, Lukic ML. An Update on the pathogenesis of Diabetes Mellitus, Department of Pathology and Medical Microbiology (Immunology Unit). Al Ain, United Arab Emirates: Faculty of Medicine and Health Sciences, UAE University; 1992.
5. Raju SM, Raju B. Illustrated medical biochemistry. 2nd ed. New Delhi, India: Jaypee Brothers Medical Publishers ltd; 2010.
6. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2010;33;Suppl 1:S62-9. doi: 10.2337/dc10-S062, PMID 20042775.
7. Jasemi SV, Khazaei H, Aneva IY, Farzaei MH, Echeverría J. Medicinal Plants and Phytochemicals for the Treatment of Pulmonary Hypertension. Front. Pharmacol. 2020; 11.
8. Oveissi V, Ram M, Bahramsoltani R, Ebrahimi F, Rahimi R, Naseri R, Belwal T, Devkota HP, Abbasabadi Z, Farzaei MH. Medicinal plants and their isolated phytochemicals for the management of chemotherapy-induced neuropathy: Therapeutic targets and clinical perspective. DARU J. Pharm. Sci. 2019; 27: 389–406.

9. Kooti W, Servatyari K, Behzadifar M, Asadi-Samani M, Sadeghi F, Nouri B, Marzouni HZ. Effective Medicinal Plant in Cancer Treatment, Part 2: Review Study. J. Evid.-Based Integr. Med. 2017; 22: 982–995.
10. O'Hagan D. Pyrrole, pyrrolidine, pyridine, piperidine and tropane alkaloids (1998 to 1999). Nat. Prod. Rep. 2000; 17: 435–446.
11. Zengin G, Guler GO, Aktumsek A, Ceylan R, Picot CMN, Mahomoodally MF. Enzyme Inhibitory Properties, Antioxidant Activities, and Phytochemical Profile of Three Medicinal Plants from Turkey. Adv. Pharmacol. Sci. 2015; 1–8.
12. Gandhi A, Dutta A, Pal A, Bakshi P. Recent trend of phytosomes for delivering herbal extract with improved bioavailability. J Pharmakon Phytochem. 2012; 1(4): 6.
13. Kalita B, Das KM, Sharma KA. Novel phytosome formulation making herbal extract more effective. J Pharm Technol. 2013; 6 (11): 1295.
14. Jadhav IA, Wadhave AA, Arsul VA, Sawarkar HS. Phytosome a novel approach in herbal drug. Int J Pharm Anal. 2014; 2(5): 478.
15. Atifa U, Sharma HK, Budholiya P, Jain P. Study of bioactive constituents and antidepressant potential of hydroalcoholic extract of Urtica urens L. in mice. Asian Journal of Pharmaceutical Education and Research. 2019; 8(3):53-60.
16. Parkhe G, Bharti D. Phytochemical investigation and determination of total phenols and flavonoid concentration in leaves extract of *Vitex trifolia* Linn. Journal of Drug Delivery & Therapeutics. 2019; 9(4-A):705-707.
17. Mishra AG, Singh R, Patil N, Parkhe G. Determination of total phenolic, flavonoid content, antioxidant and antimicrobial activity of gloriosa superb seed extract. Asian Journal of Pharmaceutical Education and Research. 2017; 6(2):12-17.
18. Kidd PM. Bioavailability and activity of phytosome complexes from botanical polyphenols: the silymarin, curcumin, green tea, and grape seed extracts. Altern Med Rev. 2009; 14(3): 226-246.
19. Hung W, Lee M, Chen F, Huang H. The condensing effect of cholesterol in lipid bilayers. BPJ. 2007; 92:3960-3967.
20. Vandijk C, Driessen A, Recourt K. The uncoupling efficiency and affinity of flavonoids for vesicles. Biochem Pharmacol. 2000; 60:1593-1600.
21. Higuchi T. Mechanism of sustained-action medication: theoretical analysis of rate of release of solid drugs dispersed in solid matrices. J Pharm Sci 1963; 52:1145-49.
22. Riaz M. Stability and uses of liposomes. Pak J Pharm Sci. 1995; 8:69-79.